Modulation of Body Composition and Immune Cell Functions by Conjugated Linoleic Acid in Humans and Animal Models: Benefits vs. Risks

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ABSTRACT: We have reviewed the published literature regarding the effects of CLA on body composition and immune cell functions in humans and in animal models. Results from studies in mice, hamsters, rats, and pigs generally support the notion that CLA reduced depot fat in the normal or lean strains. However, in obese rats, it increased body fat or decreased it less than in the corresponding lean controls. These studies also indicate that *t*10,*c*12-CLA was the isomer that reduced adipose fat; however, it also increased the fat content of several other tissues and increased circulating insulin and the saturated FA content of adipose tissue and muscle. Four of the eight published human studies found small but significant reductions in body fat with CLA supplementation; however, the reductions were smaller than the prediction errors for the methods used. The other four human studies found no change in body fat with CLA supplementation. These studies also report that CLA supplementation increased the risk factors for diabetes and cardiovascular disease including increased blood glucose, insulin, insulin resistance, VLDL, C-reactive protein, lipid peroxidation, and decreased HDL. Most studies regarding the effects of CLA on immune cell functions have been conducted with a mixture of isomers, and the results have been variable. One study conducted in mice with the purified *c*9,*t*11-CLA and *t*10,*c*12-CLA isomers indicated that the two isomers have similar effects on immune cell functions. Some of the reasons for the discrepancies between the effects of CLA in published reports are discussed. Although significant benefit to humans from CLA supplementation is questionable, it may create several health risks in both humans and animals. On the basis of the published data, CLA supplementation of adult human diets to improve body composition or enhance immune functions cannot be recommended at this time.

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CLA is a collective term for isomers of linoleic acid that have conjugated double bonds. All of the positional and geometric isomers of CLA can now be purified, and their mass spectra have been reported (1). Most of the published studies have used a mixture of CLA isomers that contained the two major forms, *cis*9,*trans*11-CLA (*c*9,*t*11-CLA) and *trans*10,*cis*12-CLA (*t*10,*c*12-CLA), and a number of minor isomers. However, the exact isomeric composition of the CLA used in many studies conducted before 1998 may not be accurate because the methylation procedures used for analysis involved heating with methanolic hydrochloric acid. That can cause interconversion of different isomers. Furthermore, the chromatographic columns used had poor isomer resolution (2).

Feeding a mixture of CLA isomers to animal models has been reported to alter chemically induced carcinogenesis (3–14), atherogenesis (15–17), diabetes (18), body composition (19–43), and immune cell functions (44–54). These studies suggest that there may be possible health benefits from CLA supplementation of animal diets. However, the data regarding most of the health benefits are controversial and also raise concerns regarding adverse effects of CLA. Only a limited number of CLA supplementation studies have been conducted in humans (55–68), and the results reported are variable. Effects of CLA on body composition in animal models, lipid metabolism, anticarcinogenic effects, and the mechanisms involved have been the topic for several recent reviews (69–73). Here, we will briefly review the effect of CLA on body composition in animal models but will focus on its effects on body composition in humans, immune functions in humans and animal models, and possible adverse effects.

EFFECT OF CLA ON BODY COMPOSITION IN ANIMAL MODELS

CLA supplementation has been found to reduce depot fat in several animal species, including mice, hamsters, rats, and pigs (19–43). Most of these experiments were conducted with growing animals by supplementing their diets with a mixture of CLA isomers ranging from 0.25 to 2.0 weight%. Effects on body composition could be noticed within 2 wk of CLA supplementation. In general, animals consumed the feed *ad libitum*, and one study found a reduction in food intake in animals fed CLA-containing diets (24). In CLA studies conducted with mice, lean body mass either increased (20), remained unchanged (22), or was not examined (25). Supplementing a mixture of CLA isomers (1% for 8 mon) to diets of mice caused a complete loss of brown adipose tissue, but also

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Abbreviations: BMI, body mass index; Con A, concanavalin A; DEXA, dual energy X-ray absorptiometry; IL, interleukin; INF, interferon; LPS, lipopolysaccharide; MLN, mesenteric lymph node; PGE_2, prostaglandin E_2; PHA, phytohemagglutinin; TNF-α, tumor necrosis factor-α.

caused 3.6- and 1.6-fold increases in liver and spleen masses, respectively (24,25). Those changes were associated with an increase in the amount of circulating insulin and a decrease in the amount of circulating leptin (23–25). In another study with mice, *t*10,*c*12-CLA (0.5% for 8 wk), but not *c*9,*t*11-CLA reduced retroperitoneal fat by 50%; however, the liver mass and liver lipids were increased by 200 and 500%, respectively, compared with the corresponding values in mice fed the control diet (43). This and other studies in mice and hamsters show that the *t*10,*c*12-CLA, but not *c*9,*t*11-CLA was the isomer responsible for reduction in adipose fat (19,26). Whether the fat is stored in liver, spleen, and muscle instead of being stored in adipose tissue or whether there is indeed a reduction in total body fat is still an open question. Risks associated with increased insulin resistance and hepatic lipid storage raise concern regarding the use of CLA to reduce adipose fat.

Supplementing the diets of Sprague-Dawley rats with a mixture of CLA isomers (3% for 3 wk) caused a 27% reduction in body fat and an 11% increase in lean body mass compared with the corresponding values in rats fed a control diet (31). In other strains of rats, results varied between the lean and obese rats. For example, in the Otsuka Long Evans Tokushima fatty rats, CLA supplementation (1% for 4 wk) reduced the perirenal, epididymal, and omental fat pads by 36, 54, and 26%, respectively, compared with the corresponding fat pads in rats fed the control diet (29); however, in Zucker obese rats, CLA supplementation (0.5% for 8 wk) caused a 15% increase in both inguinal and retroperitoneal fat pads (32). In the lean Zucker rats, CLA supplementation caused an 11 and 24% decrease in inguinal and retroperitoneal fat pads, respectively. Those results indicate that effects of CLA vary with the genotype of the animal.

The effects of CLA on body composition in pigs have been variable. CLA supplementation reduced body fat in some (34,37–40) but not in other (35,36,42) studies. One of the most likely reasons for this discrepancy was that growing pigs fed high-fat diets did not respond to CLA, whereas finisher pigs fed conventional low-fat diets did. Other reasons, including differences in the concentration of 10*t*,12*c*-CLA and the methods used to evaluate body fat, may also have contributed to these controversial results. Studies in pigs have also reported that CLA supplementation increased the saturated FA content and decreased the unsaturated FA content of adipose tissue and longissimus muscle (35,37). This improved the firmness of the meat, which may be desirable; however, it may make the meat more atherogenic to the consumer.

In conclusion, the effectiveness of CLA in altering body composition has been variable in different animal species, and risks are associated with its use. Reduction in fat mass has been attributed to increased energy expenditure and loss in excreta (21), increased adipocyte apoptosis (25), and a reduction in cell size (30). In mice fed diets containing *t*10,*c*12-CLA, the mRNA for leptin and adiponectin in the adipose tissue were decreased by 82 and 11%, respectively, compared with the corresponding values in mice fed a control or *c*9,*t*11-CLA-containing diet (43). These results, together with the reduction in circulating leptin concentrations observed in several other studies, suggest a possible role for these hormones in mediating the effects of CLA on body composition. Other details regarding the mechanisms by which CLA alters body composition can be found in a recent review (72).

EFFECT OF CLA ON BODY COMPOSITION IN HUMANS

Eight human studies have been published that report the effects of CLA on human body composition (Table 1; Refs. 55–64,68). Four of these studies were conducted in subjects with normal body weights [body mass indices (BMI) <25 kg/m^2], whereas the other four were conducted in overweight or obese subjects. The amount of CLA consumed ranged from 1.7 to 6.8 g/d; it was a variable mixture of different isomers, except for one study that used purified *t*10,*c*12-CLA (63). Only one of these studies was conducted in a metabolic unit; all of the other studies were among free-living subjects.

We conducted a 94-d study in a metabolic unit with healthy women (n = 17; mean age, 28.7 yr; BMI, 22.7 kg/m^2) (55,56). A 5-d dietary menu was used throughout the study, and all subjects participated in two daily walks of two miles each. For the first 30 d, all subjects supplemented their diets with six 1-g capsules of sunflower oil (placebo). For the next 64 d, the placebo supplement was replaced with 6 g of Tonalin for 10 women; the other 7 women continued to take the placebo capsules. Tonalin provided 3.9 g total CLA/d, with the *c*9,*t*11-CLA and *t*10,*c*12-CLA isomers each providing ~1 g; the remainder was comprised of other isomers. Body weight, fat mass (determined by total body electrical conductivity), and lean body mass did not change between days 30 and 94, the intervention period, in both groups. Energy expenditure, fat oxidation, rate of lipolysis, and FA reesterification were also not altered by CLA supplementation. No obvious health risks appeared to be associated with CLA supplementation, except a 20% increase in circulating insulin and a similar decrease in leptin concentrations (56). These changes in hormones are consistent with those discussed in animal models above and must be monitored closely in future human studies with CLA.

Another study with normal-weight subjects was conducted in Norway by Thom *et al*. (57). Men (n = 10) and women (n = 10) equally divided between the CLA and placebo groups participated in a 12-wk study. Subjects in the CLA group were administered a supplemental mixture of CLA isomers (1.8 g/d), whereas those in the control group were given a placebo (hydrogel). All subjects did standardized exercises in a gym for 90 min, three times a week. Body fat, as measured by IR interactance, decreased by 4% in the CLA group but did not change in the control group. Body weight did not change in either group. A third study with normal-weight subjects was conducted in Sweden by Smedman and Vessby (58). Men (n = 27) and women (n = 26), 23–63 yr old, were divided into two groups; one group supplemented their diets with 4.2 g/d CLA, whereas the other group supplemented with an equivalent amount of olive oil for 12 wk. The CLA used in

TABLE 1
Effect of CLA on Human Body Composition (BC)[a]

CLA type/ amount (g/d)	Time	*n*	Age (yr)	BMI (kg/m^2)	BC method	BW change (kg)	FM change (kg, cm, or %)	Comments	Reference
Sunflower oil placebo	9 wk	7	29 ± 7	22 ± 3	TOBEC	+0.5	+0.01	Diet, exercise, and all other	55
CLA mix, 3.9		10	27 ± 6	23 ± 2		– 0.2	–0.2	activity controlled in MRU.	
Hydrogel placebo	12 wk	10	28 ± 3	23 ± 3	Infrared interactance	+0.2	+0.4	Exercise 90 min, 3 times/wk. Prediction error of method >	57
CLA-mix, 1.8		10	28 ± 3	23 ± 2		–1.9	–3.4	change in BF.	
Olive oil placebo	12 wk	24	48 ± 10	25 ± 4	Skinfold	+0.2	–0.4%	% BF ↓3.8% in CLA group,	58
CLA-mix, 4.2		26	43 ± 13	26 ± 4	thickness	+0.4	–1.1%	no change in BW, BMI, or SAD.	
Olive oil placebo	28 d	Total 23	23 ± 1	25.2	DEXA	– 0.1	+0.1	No change in BW, BMI,	68
CLA-mix, 6						+0.1	+0.5	fat mass or % BF	
Placebo, unknown	6 mon	40	NA	Obese	Underwater	–2.5	–1.0	Subjects restricted food intake,	59
CLA-mix, 2.7		40		Obese	weighing	–2.5	–1.0	increased exercise.	
Olive oil placebo	12 wk	10	44 ± 13	28 ± 2	DEXA	+1.4	+1.5	Amount of exercise varied	60
CLA-mix, 1.7		12	47 ± 14	30 ± 3		–0.4	–1.2	among different groups;	
CLA-mix, 3.6		8	43 ± 10	28 ± 2		–0.4	–1.7	60% of subjects showed AE.	
CLA-mix, 5.1		11	48 ± 11	29 ± 3		–0.1	–0.4		
CLA-mix, 6.8		11	46 ± 13	30 ± 3		–0.8	–1.3		
Olive oil placebo	4 wk	10	52 ± 8	32 ± 2	SAD	–0.4	ΔSAD, 0	No change in BW, BMI, or WHR.	61
CLA-mix, 4.2		14	54 ± 6	32 ± 3		–0.3	–0.6 cm		
Olive oil placebo	12 wk	19	53 ± 10	30 ± 2	SAD + BIA	+0.1	–0.6 cm	Changes in SAD, % BF.	63
CLA-mix, 3.4		19	51 ± 7	30 ± 2		–0.5	–0.9 cm	BMI not different among the	
*t*10,*c*12-CLA, 3.4		19	55 ± 7	31 ± 3		–0.9	–0.9 cm	three groups.	

[a]Abbreviations: AE, adverse effects; BF, body fat; BIA, bioelectrical impedance analysis; BMI, body mass index; BW, body weight; DEXA, dual energy X-ray absorptiometry; FM, fat mass; MRU, metabolic research unit; SAD, sagittal diameter; TOBEC, total body electrical conductivity; WHR, waste-to-hip ratio.

that study contained ~38% of each of the *c*9,*t*11-CLA and *t*10,*c*12-CLA, with the remainder made up of other FA. At the end of the study, body weight, BMI, waist-to-hip ratio, and sagittal abdominal diameter did not change in either group. The percentage of body fat as determined by skinfold thickness decreased by 1.2% in the control group and 3.8% in the CLA group; the difference between the two groups was significant ($P = 0.05$). In the fourth study, experienced, resistance-trained men ($n = 23$) were divided into two groups; one group supplemented their diets with olive oil (9 g/d for 28 d) and the other with CLA (6 g/d + olive oil 3 g/d). Body composition as determined by dual energy X-ray absorptiometry (DEXA) showed no change in fat-free mass, fat mass, and percentage of body fat in either group (68). Thus, two of four studies with normal-weight subjects reported a reduction in body fat with CLA supplementation, but the changes observed were smaller than the prediction errors for the methods used to determine body composition.

One study with obese subjects (BMI, 30 kg/m^2) was conducted with men and women ($n = 80$) at the University of Wisconsin–Madison (59). Half of the subjects supplemented their diets with 2.7 g/d of a mixture of CLA isomers for 6 mon, whereas the other half supplemented with a placebo. Subjects in both groups were encouraged to increase their exercise level and reduce food intake. At the end of the study, body weight decreased by 2.5 kg and fat mass (underwater weighing) decreased by 1 kg in both groups. These changes could not be attributed to CLA and were most likely the result of reduced food intake and increased exercise. The second study with overweight subjects was conducted in Norway by Blankson *et al.* (60). Men and women ($n = 47$ BMI, 28–30) were divided into five groups and received different amounts of CLA (0, 1.7, 3.4, 5.1, and 6.8 g/d) supplements for 12 wk. The placebo used was olive oil (9 g/d). CLA supplements provided equal amounts of the *c*9,*t*11-CLA and *t*10,*c*12-CLA, and the total fat supplement in all groups was comparable to the placebo group. Subjects in all groups were asked to participate in either light or intensive exercise. The number of subjects participating in two levels of exercise varied among different CLA groups. Fat mass, as determined by DEXA, decreased significantly in the groups taking CLA supplements of 3.4 or 6.8 g/d, but not in those taking 1.7 or 5.1 g/d. The mean amount of fat lost was 1.7, 0.4, and 1.3 kg in those taking 3.4, 5.1, and 6.8 g CLA, respectively. The authors claim that CLA intake of 3.4 g/d reduced body fat. This conclusion was weakened because the decrease in body fat was not significant in the group administered 5.1 g CLA. Furthermore, the changes in fat mass seen were within the prediction error for DEXA (1.65 kg). Lean body mass increased (0.88) significantly only in the group administered 6.8 g CLA. This is the group that reported maximum increase in the number of hours spent with intensive exercise. Those confounding variables make it difficult to distinguish whether the increase in lean body mass was due to increased exercise or the CLA supplement. Of the 60 study

participants, 36 exhibited side effects, including fatigue, gastrointestinal problems, and relapse of asthma. The authors claimed that there was no difference in adverse effects between the control and CLA groups. However, with the small number of subjects, it is difficult to prove or disprove this claim; it is obvious, though, that the smallest number of adverse effects occurred in the control group and the greatest number in the group with the highest supplement of CLA. Future studies should address this safety issue.

Two studies with obese subjects were conducted in Sweden (61,63). In one of these studies (61), 24 abdominally obese men (39–64 yr; BMI, 32 kg/m^2) participated in a double-blind randomized control trial for 4 wk. Fourteen men were given 4.2 g CLA/d, and 10 men received a placebo (olive oil). The CLA used contained ~38% of each of the *c*9,*t*11-CLA and *t*10,*c*12-CLA, with the rest made up of minor CLA isomers and other FA. At the end of 4 wk, the mean sagittal abdominal diameter in the CLA group decreased from 29.5 to 28.9 cm, whereas it remained unchanged at 29.2 cm in the control group. In the second study (63), abdominally obese men with metabolic syndrome (n = 60; 35–65 yr) were divided into three groups and received 3.4 g/d of FA supplements for 12 wk. The supplements used were olive oil (placebo), a mixture of CLA isomers as used in their earlier study, or CLA enriched with the *t*10,*c*12-CLA isomer alone. Bioelectrical impedance was used to calculate total body fat, and sagittal abdominal diameter was used to approximate central fat. Total adiposity and BMI were not altered with either of the CLA supplements, but central fat tended to decrease with both CLA supplements. The changes seen were smaller than the prediction error for the methods used.

Collectively, results from human studies regarding the effects of CLA on body composition were quite variable. Four of the eight studies indicated a possible reduction in body fat with CLA supplementation, but the other four reported no change. If the reported changes in body fat resulted from CLA intake, then discrepancies among studies may be the result of differences in the isomer composition or amount and duration of CLA supplement. It is also possible that these changes were the result of confounding variables, including food, exercise, and the prediction errors for the methods used. Long-term controlled studies are required to determine whether indeed CLA intake reduces body fat in humans. Before such studies are undertaken, it is important to address the potential risks associated with CLA supplementation.

EFFECT OF CLA ON IMMUNE FUNCTIONS IN ANIMAL MODELS

There are less than a dozen published studies examining the effects of CLA supplementation on immune cell functions in animal models (Table 2). The amount of CLA fed ranged from 0.1 to 1.5 weight% of the diet, and the feeding duration ranged from 2 to 8 wk. The CLA mixture used was reported to contain ~40% each of the *c*9,*t*11 and *t*10,*c*12 isomers with the remainder made up of the minor components. Accuracy of the isomer composition reported may be debated for some of the studies. Growing animals were used in most of the studies and

TABLE 2
Effect of CLA on Immune Cell Functions in Animal Models[a]

Index	CLA amount/type (wt% of diet)	Time (wk)	Species	*n*/group	Effect	Comments	Reference
DTH	0.5, CLA-mix	4	Rat	7	Increased	Foot-pad swelling 24 h post-PHA	45
DTH	1.0, CLA-mix	8	Mice	10	NC	Urd incorporation into ear 16 h post-DFNB	47
Listeria resistance	0.5, CLA-mix	4	Mice	4–5	NC	Infer no decrease in cellular immunity	48
SPC proliferation	1.0, CLA-mix	8	Mice	10	Increased or NC	Responses varied to PHA and Con A	47
SPC proliferation	0.1, 0.3, or 0.9, CLA-mix	6	Mice	8	NC	PHA, Con A, and LPS used for stimulation	46
SPC proliferation	0.5, purified 9/11 and 10/12	12	Mice	12	NC	Con A and LPS used	54
IL-2 secretion	1.0, CLA-mix	8	Mice	10	Increased in young not old	Con A: 24 h	47
IL-2 secretion	0.1, 0.3, or 0.9, CLA-mix	6	Mice	8	NC	Con A: 24 h	46
IL-2 secretion	0.5, purified	8	Mice	12	NC	Con A: 4, 24, 48 h	54
IL-4 secretion	0.5, purified	8	Mice	12	Decreased by both isomers	Con A: 4, 24, 48 h	54
IL-6 secretion	0.5, purified	8	Mice	12	Increased by both isomers	LPS: 4, 24, 48 h	
TNF and secretion	0.5, purified	8	Mice	12	Increased by both isomers	LPS: 4, 24 , 48 h	54
Serum Ab	0.5 or 1.0, CLA-mix	3	Rat	5	Increased IgA, IgG, IgM Decreased IgE	Similar effect seen in splenic and MLN cells	49
Serum Ab	0.05–0.5, CLA-mix	3	Rat	5	NC	*In vitro* CLA decreased Ig secretion by SPC	50

[a]Abbreviations: Ab, antibody; Con A, concanavalin A; DFNB, difluoronitrobenzene; DTH, delayed-type hypersensitivity skin response; IL, interleukin; LPS, lipopolysaccharide; MLN, mesenteric lymph node; NC, no change; PHA, phytohemagglutinin; SPC, splenocyte; TNF, tumor necrosis factor; Urd, deoxyuridine; 9/11, *cis*9,*trans* 11-CLA; 10/12, *trans*10,*cis*12-CLA.

food intake was not controlled. Effects of CLA varied from stimulation to inhibition, depending on the function examined or even for the same function examined in different studies. Here, we will discuss the *in vivo* and *ex vivo* effects of CLA on immune status and response in animal models.

Three studies examined the effects of CLA supplementation on splenocyte proliferation in response to T- and B-cell mitogens (46,47,54). In the first study, 0.3 or 0.9 weight% CLA was fed for 3 or 6 wk to 8-wk-old, female Balb/c mice. After 3 wk, splenocyte proliferation in response to the T-cell mitogen, phytohemagglutinin (PHA), was 146 and 192% in the 0.3 and 0.9 CLA groups, respectively, compared with the control group (safflower oil), but at 6 wk, the three groups did not differ (46). CLA supplementation did not alter splenocyte proliferation in response to another T-cell mitogen, concanavalin A (Con A), or the B-cell mitogen, lipopolysaccharide (LPS), after 3 or 6 wk of supplementation. The second study (47) used 4- or 24-mon-old C57BL/6N mice; their diets were supplemented with 0 or 1% CLA for 8 wk. The proliferation of splenocytes isolated from the young mice fed the CLA-containing diet was approximately twice that in the control mice when Con A concentrations of 0.5 and 5.0 were used; at a Con A concentration of 1.5 mg/L, however, the difference in proliferation between the control and CLA groups was not significant. In the old mice, the splenocyte proliferation in the CLA group was twice that of the control group at a Con A concentration of 1.5 mg/L, but at the other two concentrations, the CLA and control groups did not differ. Proliferation in response to PHA was tested at concentrations of 5, 20, and 40 mg/L. It was not different between the control and CLA groups at all PHA concentrations in both the young and old mice, except at 40 mg/L, where it was significantly greater (50% more) for the young mice fed the CLA diet. Similarly, results regarding splenocyte proliferation in response to the B-cell mitogen, LPS, varied with its concentration. In the third study, 8-wk-old female mice were fed diets containing purified isomers of CLA (*c*9,*t*11- or *t*10,*c*12-CLA, 0.5% for 8 wk); splenocyte proliferation in response to several different concentrations of Con A and LPS did not differ between the control and CLA groups (54). In view of the variability in the data from these studies, it is difficult to determine whether CLA indeed enhanced T- or B-cell proliferation in response to mitogens.

The above-mentioned three studies also examined *ex vivo* interleukin (IL)-2 production by splenocytes stimulated with Con A. In one study (46), IL-2 production after 3 wk of CLA supplementation was 126, 230, and 192% in the 0.1, 0.3, and 0.9% CLA groups, respectively, compared with the corresponding value in the control group; at 6 wk, the four groups did not differ. In the other study, IL-2 secretion was significantly increased (188% of control) after CLA supplementation in young but not old mice (47). Supplementation of purified isomers of CLA did not alter IL-2 secretion when tested at 4, 24, and 48 h in culture with two different concentrations of Con A (54). Secretion of the Th-2 cytokine, IL-4, was not different among the three groups at 4 h after treatment with Con A, but it was significantly reduced (30–70%) by both isomers at 24 and 48 h. The period of time for which the splenocytes were treated with the mitogens seems critical in detecting the effects of CLA on the secretion of Th2 and inflammatory cytokines. These studies also reported that CLA supplementation had no effect on lymphocyte cytotoxicity (46) and natural killer cell (47) activities. Results from another study showed that 0.5% CLA supplementation, for 4 wk, did not alter *in vivo Listeria* infection (48). CLA feeding increased foot swelling in response to PHA in rats (45), but did not alter the ear responses after 2,4-dinitrofluorobenzene in mice (47). Whether these discrepancies are due to the lack of a CLA effect on lymphocyte functions or to the differences in experimental protocols cannot be established from the information available.

Results from CLA studies on lymphocyte functions in rats are also variable. Supplementing the diets of male 4-wk-old Sprague-Dawley rats with 0.5 or 1.0% CLA for 3 wk led to a significant (6 and 22%) reduction, respectively, in the *in vitro* secretion of IgE, by mesenteric lymph node (MLN) cells and significant increases (200–700%) in the secretion of IgG, IgA, and IgM compared with the corresponding values in the rats fed control diet (49). The effect of CLA supplementation on the secretion of Ig by splenocytes and on their serum concentrations was much smaller than that seen with MLN. The ratio between the CD4+ and CD8+ cells in the MLN was not altered by CLA supplementation, suggesting that the effect of CLA may be directly on the B cells. In a subsequent study (50), the same authors examined the effects of a range of CLA concentrations (0.0, 0.05, 0.10, 0.25, and 0.50%) on both the serum levels of Ig and their *in vitro* secretion by splenocytes. *Ex vivo* secretion of IgA, IgG, and IgM increased as the concentration of CLA in the diet was increased with the maximum (two- to threefold increase) attained at 0.1 or 0.25%. However, there was no effect on the serum concentration of the three Ig at all CLA concentrations tested. Results of the first study from this group showed a twofold increase in serum concentration of IgG with a CLA dose of 0.5%, whereas in the second study, this dose of CLA had no effect on serum IgG. Rats of the same strain and age and the same feeding duration were used in the two studies; thus, these factors could not account for the discrepancy between the results. The authors did not comment on this discrepancy but suggested that CLA supplementation may reduce the risk of food allergies. Antibody response in rats and chicks to BSA and sheep red blood cells was not affected by CLA feeding (45). Overall, these results do not support the claim that CLA feeding improves antibody response in animals.

Only a few studies have examined the effects of CLA on monocyte/macrophage cell functions. Peritoneal macrophages isolated from the rats fed CLA exhibited more than twice phagocytic activity compared with those isolated from rats fed the control diet (45). In another study, addition of CLA (1%) to a soybean oil-based rat diet caused a >50% reduction in *ex vivo* secretion of IL-6 by resident peritoneal macrophages, but it had no effect when added to a menhaden oil-based diet (51). In this study, CLA feeding also decreased the basal but not the

LPS-simulated secretion of tumor necrosis factor-α (TNF-α) in both the soybean oil- and menhaden oil-based diets. CLA feeding had no effect on the *ex vivo* production of IL-1 and prostaglandin (PG)E_2 by rat peritoneal macrophages (51) and mouse splenocytes (47). Supplementing the diets of mice with purified isomers of CLA had similar effects on the *ex vivo* secretion of inflammatory cytokines (54). Secretion of TNF-α by the cultured splenocytes decreased between 4 and 48 h, whereas that of IL-6 increased. TNF-α secretions were significantly increased (>30%) by both the *c*9,*t*11- and *t*10,*c*12-CLA isomers at 4 h, but not at 24 or 48 h, after treatment with LPS. IL-6 secretion was increased in both CLA groups by 30–50% at 24 and 48 h, but not at 4 h. Differences in the effects of CLA on the secretion of inflammatory cytokines between mice and rats may be due to species differences, different cell types, or the diets; however, they may also be due to the duration of cell culture. We found that the period of time for which cells were cultured was critical in detecting CLA effects on the inflammatory and Th2 cytokines.

In summary, the effects of CLA on immune functions in animal models have been variable. Differences may be due to a number of factors, including species, strain, age, and health of the animals used, FA and antioxidant nutrient composition of the diets, feeding regimen, amount and duration of CLA supplementation, culture conditions, including the type and amount of serum and the duration of the cell culture. None of the animal studies reported here cultured cells in autologous sera, and most examined the response variables at a single time point. Results from one study that used multiple time points and mitogen concentrations indicated that the two isomers had similar effects on a number of immune cell functions; neither of the isomers altered splenocyte proliferation or production of the Th1 cytokine IL-2; both isomers decreased production of the Th2 cytokine IL-4 and increased production of the inflammatory cytokines TNF- and IL-6. Further studies with chemically defined CLA isomer mixtures and standardized experimental protocols are required to resolve the controversies regarding the effects of CLA on immune cell functions.

EFFECT OF CLA ON IMMUNE CELL FUNCTIONS IN HUMANS

There are only four published reports in which the effects of CLA supplementation on human immune cell functions were examined (Table 3). The first was a metabolic unit study that we conducted (65,66). As discussed above, this study was conducted with 17 healthy women. For the first 30 d of the study, all subjects consumed a basal diet supplemented with sunflower oil capsules (6 g/d). The supplement for 10 women was replaced with Tonalin (6 g/d) for study days 31–94, whereas for the remainder 7 women, a placebo supplement was used throughout the study. Tonalin supplement provided 3.9 g CLA/d; it was comprised of *t*10,*c*12-CLA (22.6%), *c*11,*t*13-CLA (23.6), *c*9,*t*11-CLA (17.6), *t*8,*c*10-CLA (16.6), and other isomers (19.6). Immune cell functions were evaluated by using fasting blood samples drawn three times before the start of CLA supplementation (study days 15, 22, and 29) and three times at the end of CLA supplementation (study days 78, 85, and 92). Delayed hypersensitivity skin response to a battery of seven recall antigens was determined on study days 30 and 90. All subjects were immunized with influenza vaccine on study day 65, and the serum antibody titers were determined using the blood samples drawn on study day 65 (preimmunization) and 92 (postimmunization).

The total CLA concentration in circulating white blood cells (WBC) on study day 90 increased eightfold compared with the concentration on study day 30 (0.12–0.96% of total FA); however, it did not significantly alter the concentration of other FA. CLA supplementation did not alter the number of circulating total WBC, lymphocytes, granulocytes, monocytes, or the various subsets of lymphocytes. It had no effect on proliferation of lymphocytes cultured with PHA, Con A, or influenza vaccine, *in vitro* production of cytokines [IL-2 , interferon (INF)-γ, IL-1, and TNF-α], or the production of inflammatory eicosanoids, PGE_2 and leukotriene B_4, when cells were cultured in medium containing 10% autologous sera. Serum influenza antibody titers and delayed hypersensitivity skin response were also not affected by CLA supplementation. Overall, results of this study suggest that short-term supplementation with a modest level of CLA to healthy adult women had no beneficial effect on human immune response. This is consistent with the effects on body composition and energy metabolism discussed earlier. Results from another study indicated no change in serum concentrations of TNF-α and IL-6 when overweight/obese volunteers supplemented their diets with CLA (3.4 g/d, isomer mixture or purified *t*10,*c*12-CLA for 12 wk) or an equivalent amount of olive oil (64). In a study with experienced resistance-trained men, CLA supplementation (CLA mixture 6 g/d for 28 d) caused a 25% decrease in the ratio of circulating neutrophils to lymphocytes, whereas the ratio in the olive oil-supplemented subjects did not change (68). From the data available, it is not possible to determine whether the reduction in this ratio was due to a decrease in neutrophil numbers or an increase in lymphocyte numbers. In this study, CLA supplementation also did not significantly affect gains in bench or leg press. Whether higher amounts or longer duration of CLA supplementation would affect human immune cell functions remains to be determined; however, increasing the duration may also increase the health risks.

Another human study examined the effects of two different mixtures of CLA isomers (80:20 or 50:50) on antibody response to hepatitis B vaccination, delayed hypersensitivity skin response, and other indices of immune cell functions in healthy men (67). The total amount of CLA supplemented was 1.7 g/d for 84 d. CLA supplementation did not alter delayed hypersensitivity skin response or any of the other indices of immune cell functions tested. Mean antibody titers against hepatitis B did not differ among the three groups; however, the number of subjects attaining antibody titers >10 IU/L compared with the number of subjects with antibody titers <10 IU/L was significantly ($P < 0.05$) greater in the 50:50 group than in the 80:20 or placebo group. The validity of the claim that such arbitrary titers are seroprotective may be questionable, particularly when they are only two- to three-

TABLE 3
Effect of CLA on Human Immune Cell Functions[a]

Index	CLA (g/d)	Time (wk)	Subject type (n)	Effect	Comments	Reference
DTH to 6 Ag	3.9, CLA-mix	9	Healthy women CLA (10) Placebo (7)	↑	No difference between placebo and CLA	65
Serum Ab to influenza	3.9, CLA-mix	9	Healthy women CLA (10) Placebo (7)	↑	No difference between placebo and CLA	65
No. of circulating WBC	3.9, CLA-mix	9	Healthy women CLA (10) Placebo (7)	NC	NC in total or subsets of WBC or lymphocytes	65
PBMNC proliferation	3.9, CLA-mix	9	Healthy women CLA (10) Placebo (7)	NC	Cultured in autologous sera and PHA or influenza Ag	65
Secreted IL-1, IL-2, TNF2	3.9, CLA-mix	9	Healthy women CLA (10) Placebo (7)	NC	Cultured in autologous sera and PHA or LPS	65
Intracellular IL-2, IFN, and TNF	3.9, CLA-mix	9	Healthy women CLA (10) Placebo (7)	NC	Flow cytometry	65
Secretion of PGE_2 and LTB_4	3.9, CLA-mix	9	Healthy women CLA (10) Placebo (7)	NC	Cultured in autologous sera and LPS	65
Serum Ab to HepB	1.7, 50:50 or 80:20 mix of 9/11:10/12	12	Healthy men (25/group)	?	Mean antibody titer and number of responders not different; twice as many subjects with Ab > 10 IU/L in 50:50 than other two groups; infer 10/12 increased HepB response. Many other indices did not change.	67
Serum TNF and IL-6	3.4, CLA-mix or purified 10/12	12	Metabolic syndrome men, 19/group	NC	Many aspects of glucose and lipid metabolism deteriorated by 10/12.	64
Neutrophil/lymphocyte ratio	6.0, CLA-mix	4	Resistance-trained men, 11 or 12/group	↓25%	No change in placebo group.	68

[a]Ag, antigen; HepB, hepatitis B; LTB_4, leukotriene B_4; PBMNC, peripheral blood mononuclear cells; PGE_2, prostaglandin E_2; WBC, white blood cells; for other abbreviations see footnotes to Tables 1 and 2.

fold higher than the preimmunization titers. Because the 50:50 mixture contained more of the *t*10,*c*12-CLA isomer than the 80:20 mixture, the authors concluded that *t*10,*c*12-CLA enhanced the antibody response to hepatitis B, whereas the *c*9,*t*11-CLA did not alter that response. Those results are not consistent with the observed effects of CLA on influenza antibody titers (65). Whether this is antigen specific, sex specific, or the result of differences in CLA isomers used must be resolved in future studies.

ADVERSE HEALTH EFFECTS OF CLA

The adverse effects of CLA supplementation of animal and human diets are summarized in Table 4. Although there is a reduction in adipose fat in several animal species, there is a concomitant increase in liver, spleen, and muscle fat in the animal models. The consequences of increased fat on the functions of these organs must be monitored. Studies in pigs indicate that CLA supplementation increased the saturated fat content of the muscle; this improved the firmness of the meat, but also made it more atherogenic. Results from studies in mice and humans indicate an increase in the concentration of blood sugar and insulin, insulin resistance, VLDL, and a reduction in blood leptin and HDL. In overweight human subjects, *t*10,*c*12-CLA caused a several-fold increase in lipid peroxidation and a twofold increase in serum C-reactive protein. These changes indicate that CLA supplementation could promote an increased tendency toward diabetes and cardiovascular disease. Against this background of risks and no known benefits of CLA for humans, the wisdom of its supplementation to humans is questionable. Furthermore, its supplementation to animal diets may also increase health risks to humans who consume foods from such animals. It seems that most of the health risks are attributable to the *t*10,*c*12-CLA, and that the *c*9,*t*11-CLA may be safe for human consumption. If *c*9,*t*11-CLA is found to have anitcarcinogenic or other health benefits in humans as claimed in animals, future studies should concentrate on this isomer. The risks and benefits of many other CLA isomers are unknown and may be of interest.

CONCLUSIONS AND FUTURE DIRECTIONS

Results from animal studies indicate that the *t*10,*c*12-CLA isomer reduced body fat in several different species, but raise safety concerns dealing with the deposition of fat in other tissue, altering the FA profiles of the tissues, as well as serum insulin and leptin concentrations. Although the effects of CLA on immune cell functions have been quite variable, the two isomers seem to have similar effects. This is in contrast to the differences in their effects on body composition. Results from human studies regarding the effects on both body composition and immune cell functions have been variable. These studies also raise safety concerns, in particular con-

TABLE 4
Adverse Effects of CLA in Animals and Humans[a]

Index	CLA amount/type	Duration	Species	Effect	Comments	Reference
Liver weight	1%, CLA-mix	4 d to 8 mon	Mice	3.6 × control	May impair liver function	25
Spleen weight	1%, CLA-mix	4 d to 8 mon	Mice	1.6 × control	May impair spleen function	25
Liver weight	0.5%, CLA 9/11	8 wk	Mice	Not different than control	May impair liver function	43
	0.5%, CLA 10/12	8 wk	Mice	2 × control		
Liver lipids	0.5%, CLA 10/12	8 wk	Mice	5 × control	Fat stored in liver instead of AT	43
Serum insulin	1.0%, CLA-mix	4 d to 8 mon	Mice	4 × increase in fasting insulin 8 × increase in fed state	Indicated increased insulin resistance	43
Serum leptin	1.0%, CLA-mix	4 d to 8 mon	Mice	↓49% (fasting) ↓79% (fed)	Leptin infusion Corrected insulin resistance	25
Muscle FA composition	0.25, 0.5, 1.0 or 2%, CLA-mix	48 d	Pigs	Linear increase with increased CLA in 16:0 and 18:0 content	This increased meat firmness but will have adverse health effects	36
Muscle FA composition	1%, CLA-mix	7 wk	Pigs	Increased saturated FA	This increased meat firmness but will have adverse health effects	35
Fat pad weights	0.5%, CLA-mix	5 wk	Obese Zucker rats	↑15% (obese) ↓5% (lean)	Did not reduce fat in obese rats	32
Serum insulin	3.9 g, CLA-mix	9 wk	Healthy women	↑20% (day 63)	Increased insulin resistance	56
Serum insulin	3.4 g, CLA-mix or purified 10/12	12 wk	Obese men	3 × increase with 10/12 than placebo	Increased insulin resistance or mix	63
Fasting glucose	3.4 g, CLA-mix or purified 10/12	12 wk	Obese men	↑4% with 10/12	Decreased insulin sensitivity	63
Serum HDL	3.4 g, CLA-mix or purified 10/12	12 wk	Obese men	↓4% with 10/12	Lipid profile changed	63
Serum VLDL	3.4 g, CLA-mix or purified 10/12	12 wk	Obese men	↑11% with 10/12	Favored CVD	63
Lipid peroxidation	3.4 g, CLA-mix or purified 10/12	12 wk	Obese men	↑578% in urinary 8-Iso, PGF_2 with 10/12	Increased lipid peroxidation increases CVD	64
Serum CRP	3.4 g, CLA-mix or purified 10/12	12 wk	Obese men	↑110% with 10/12, ↑41% with mix	Increased inflammation	64

[a]Abbreviations: AT, adipose tissue; CRP, C-reactive protein; CVD, cardiovascular disease; PGF_2, prostaglandin F_2.

cerning glucose and lipid metabolism as well as lipid peroxidation. Controlled studies with purified isomers of CLA should be conducted to determine which isomer(s) may be responsible for health benefits as well as risks to human health. Results will have to be confirmed independently in different laboratories. Dose–response studies should be conducted to determine the minimum concentration of CLA required to produce the desired effects. The amounts of CLA that have been effective in changing body composition in growing animals would be 30–60 g/d for a 60-kg person, and thus could not be considered nutritional. The most urgent issue is to determine the benefits from CLA supplementation to humans as well as the risks associated with it. To avoid risks associated with high concentrations and long duration of CLA intake, it would be preferable to conduct initial studies with nonhuman primates. Once this information is available, future studies will have to determine the mechanisms by which CLA alters human physiologic functions.

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